DISCOVERY

Effects of nickel toxicity and tolerance in seedling growth of *Lycopersicon esculentum* Mill. and *Helianthus annuus* L.

Shumaila Fazal[1], Muhammad Zafar Iqbal[2], Muhammad Shafiq[3]

To Cite:
Fazal S, Iqbal MZ, Shafiq M. Effects of nickel toxicity and tolerance in seedling growth of *Lycopersicon esculentum* Mill. and *Helianthus annuus* L.. *Discovery* 2023; 59: e63d1242

Author Affiliation:
[1]Department of Botany, University of Karachi, Karachi, 75270, Pakistan
[2]Department of Botany, University of Karachi, Karachi, 75270, Pakistan
Email: mziqbalbotuokpk@yahoo.com
[3]Department of Botany, University of Karachi, Karachi, 75270, Pakistan
Email: shafiqeco@yahoo.com

Peer-Review History
Received: 20 April 2023
Reviewed & Revised: 24/April/2023 to 04/May/2023
Accepted: 08 May 2023
Published: June 2023

Peer-Review Model
External peer-review was done through double-blind method.

Discovery
pISSN 2278–5469; eISSN 2278–5450

© The Author(s) 2023. Open Access. This article is licensed under a Creative Commons Attribution License 4.0 (CC BY 4.0)., which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

DISCOVERY SCIENTIFIC SOCIETY

ABSTRACT

The impact of nickel salt on the seedling development of two important agriculture crops viz. *Lycopersicon esculentum* and *Helianthus annuus* belonging to family Solanaceae and Compositeae were observed. A significant reduction in seedling and root length and leaves number of *L. esculentum* with nickel treatment at higher concentrations 75 and 100 ppm. At the level of 100 ppm or 150 ppm nickel was found phytotoxic and harmful to shoot growth of *L. esculentum* and *H. annuus* as compared to control. There were no significant effects on leaf area of both plant species by Nickel at 25-100 ppm. The treatments of nickel at similar concentrations decrease seedling growth and development performance of *H. annuus*. Meanwhile, experiment also showed maximum decrease in seedling fresh weight of *L. esculentum* and *H. annuus* with nickel at 150 ppm. It was also witnesses that a gradual increase in nickel concentrations decreases in seedling dry weight for *L. esculentum* and *H. annuus*. Nickel treatment at 25, 50, 100 and 150 ppm concentration influenced on the seedling tolerance indices of *L. esculentum* and *H. annuus*. *L. esculentum* showed low percentage of tolerance (51.92 %) to nickel treatment at 150 ppm in *H. annuus.* Whereas, the lowest percentage of tolerance (44.03 %) to nickel treatment at 150 ppm was found in *L. esculentum* as compared to control. The result of this study confirm that nickel treatment consistently contributes the high level of toxicity to seedling of *H. annuus* than *L. esculentum.*

Keywords: Nickel toxicity, *Lycopersicon esculentum* Mill., *Helianthus annuus* L.

1. INTRODUCTION

Nickel released in environment due to industrial processes and widespread distribution may be exposed to population, food and toxic to plants. The effects of nickel on barley, wheat, rape, lettuce, rye grass, bean, rice and in agricultural soil were observed (Davis and Beckett, 1978; Khalid and Tinsley, 1980; Liibben and Saurebeck, 1991; Holmgren et al., 1993). High concentrations of Ni lead to reduction in minerals, water uptake and alteration in root cell division (Sen and Bhattacharyya, 1994; Ma et al., 2009; Pavlova, 2017). The treatment of 8 mM Ni

almost completely inhibited the hypocotyl elongation of *Alyssum murale* and *Alyssum markgrafii* (Pavlova et al., 2018). Ni at higher level in tissues of *Salvinia minima* Baker damage photosynthesis (Fuentes et al., 2014).

Nickel metal is a nutritionally required for animal, microorganisms, plants and too little or too much becomes toxic for various plant species (Cempel and Nikel, 2006; Demirezen et al., 2007; Chamon et al., 2009). Nickel released to environment from fossil fuel burning, industrial wastes, stainless steel manufacturing, rechargeable batteries and coinage and fertilizer application. The soils pollution due to metal is becoming a serious environmental problem increasingly over the last century (Gerhardt et al., 2009; Ruiz et al., 2009). Soil contamination in excess level of any element reply toxic response to biota and produce an unacceptable environmental risk to agricultural products (Zhang and Shan, 2000; Adriano, 2001; Vangronsveld et al., 2009; Zhu et al., 2014).

The indiscriminate discharge of metal pollutants due to industrial and anthropogenic activities is polluting the city environment of Karachi, Pakistan. These pollutants especially heavy metals individually and in a combination cause greater effects to plants. Among the heavy metals, nickel production is an important source of environmental pollution from industries and metal mining activities. Plants are vulnerable to plant (Khan et al., 2022). Crop production is markedly reduced by metal pollution. The level of heavy metals e.g., nickel is increasing in the environment due to anthropogenic and industrials activities and is an ecological threat to plants growth. Tomato is a type of fleshy juicy fruit, usually red in color and used in salad and sauces. The seeds of sunflower are a good source of cooking oil as compare to other vegetable oil and animal fats. The research was conducted to evaluate the effects of nickel on the growth performance of two an important agriculture food crops viz., sunflower (*Helianthus annuus*) and tomato (*Lycopersicon esculentum*). The present work was also calculating the tolerance to nickel when treated at different concentrations by comparing to control.

2. MATERIALS AND METHODS

The seedling growth experiment was conducted in sand culture using disposable plastic cup of Pepsi in green house of University of Karachi Campus. The sand was collected from the construction site of the Karachi University and sieved through 0.05 mm sieve plate. This was washed 2-3 times with tap water and later on washed with distilled water. The sand was also washed with 5% HCl to remove any types of impurities from the soil. The washed sand was then filled by 2/3 in disposable plastic Pepsi cans. The pots were placed in tubs and apply water for 2-3 days. A uniform size seedling of tomato and sun flower were transferred in Pepsi plastic cup for nickel treatment using different concentrations (0, 25, 50, 100 and 150 ppm) as nickel sulphate. The experiment was lasted for 28 days. The phenological appearance of seedlings of both plant species was noted on weekly basis. The irrigation was made as per requirements with the tap water. The Hoagland solution was also given with the three to four days of time interval for the supply of complete nutrition to the seedlings. The experiment was completely randomized and consists of five treatments with replicated as five times. The harvested seedlings from the pot and morphological quantitative parameters after four weeks were noted. The seedlings were dried in oven at 80° C for 24 hours.

Leaf area was calculated by the formulae (Eq. 1).

$$Leaf\ area = leaf\ length\ X\ leaf\ breadth\ X\ \frac{2}{3}\ (Eq = 1)$$

Root / shoot ratio fresh and dry weight was found by the following formula, respectively (Eq. 2-3).

$$Root\ /\ shoot\ ratio\ fresh\ weight = \frac{root\ fresh\ weight}{leaf\ fresh\ weight + shoot\ fresh\ weight}\ (Eq.2)$$

$$Root\ /\ shoot\ ratio\ dry\ weight = \frac{root\ dry\ weight}{leaf\ dry\ weight + shoot\ dry\ weight}\ (Eq.3)$$

Total seedling fresh and dry weight was determined according to following formulae (Eq. 4-5).

$$Total\ seedling\ fresh\ weight = Leaf\ fresh\ weight + shoot\ fresh\ weight + root\ fresh\ weight\ (Eq.4)$$

$$Total\ seedling\ dry\ weight = Leaf\ dry\ weight + shoot\ dry\ weight + root\ dry\ weight\ (Eq.5)$$

The tolerance indices (TI) percentage was done as per the formula (Eq. 6).

$$TI = \frac{mean\ root\ length\ of\ metal\ treated\ seedlings}{mean\ root\ length\ in\ without\ metal\ treated\ seedlings} \times 100\ \ (Eq.6\ )$$

The obtained data on plant growth variable were statistically analysed subject to get ANOVA and DMRT for level of significance on personnel computer using COSTAT version-3 software.

3. RESULTS

The nickel treatment with varying concentration (0, 25, 50, 100 and 150 ppm) of nickel sulphate affected seedling growth and yield of sun flower (*Helianthus annus*) and tomato (*Lyopersicon esculentum*). Nickle treatment at all level (25, 50, 100, 150 ppm) did not show any significant influence on leaf growth of *L. esculentum* and *H. annuus*. Nickel treatment at 50 ppm treatment showed significant ($p<0.05$) reduction in shoot and seedling size of *H. annuus*. Nickel treatment at 100 ppm significantly ($p<0.05$) decreased root growth of *H. annuus* (Figure 1). The effect of nickel treatment was found more in shoot than root growth for *H. annuus* seedlings. The nickel treatment at 25 ppm reduce root, shoot and leaves fresh and dry weight of *H. annuus* (Figure 2).

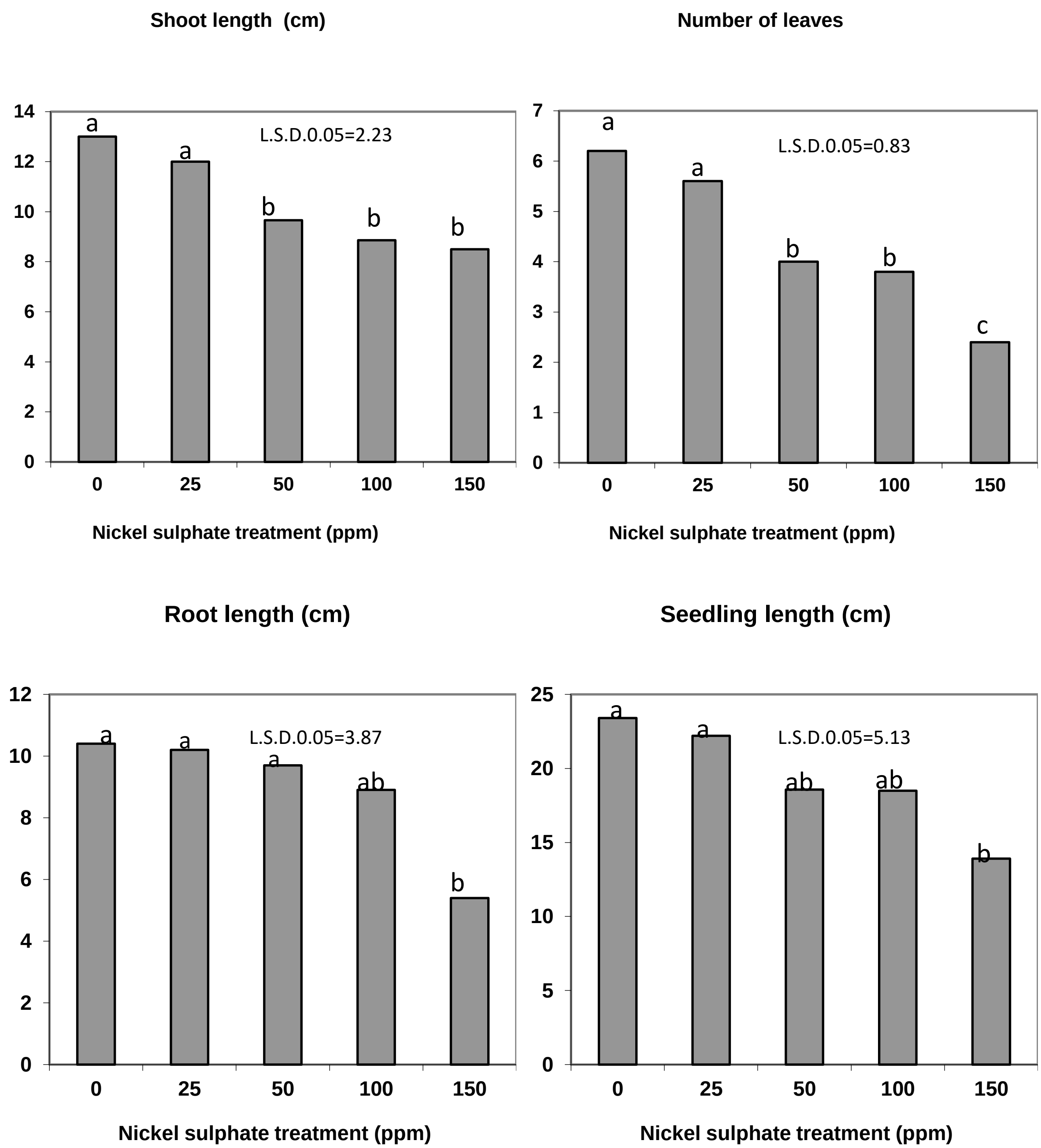

Figure 1 Effects of different concentration (0, 25, 50, 100, 150 ppm) of Nickel sulphate (Ni SO_4) on shoot, root, seedling length and number of leaves of *Helianthus annuus.* Statistical significance determined by analysis of variance. Numbers followed by the same letters in the same bar not significantly different ($p<0.05$) according to Duncan's multiple range tests

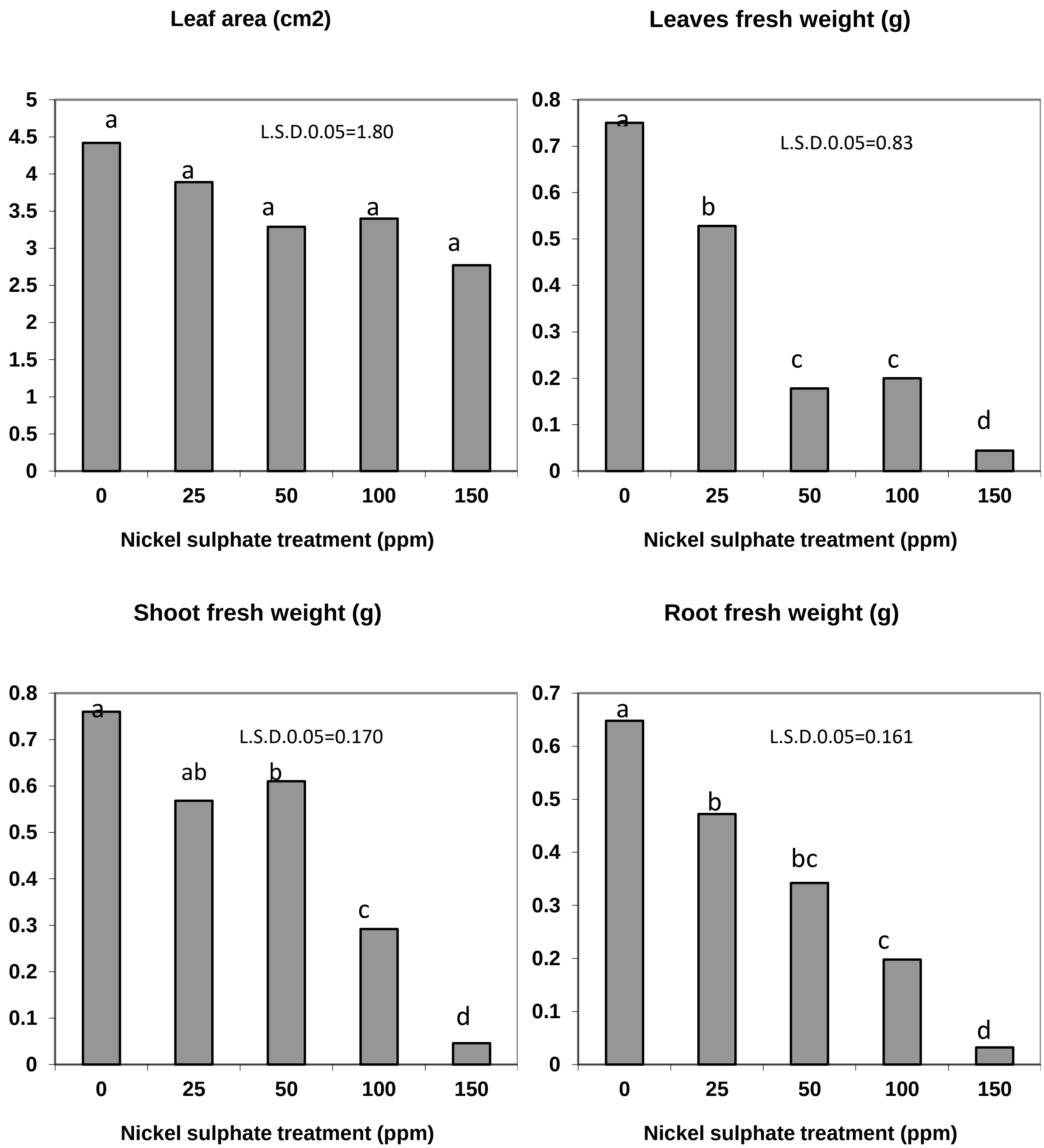

Figure 2 Effects of different concentration (0, 25, 50, 100, 150 ppm) of Nickel sulphate (Ni SO_4) on leaf area (cm^2), leaves, shoot and root fresh weight (g) of *Helianthus annus.* Statistical significance determined by analysis of variance. Numbers followed by the same letters in the same bar not significantly different ($p<0.05$) according to Duncan's multiple range tests

It was observed that nickel treatment at 25 ppm reduced number of leaves in *H. annuus*. Seedling growth performance of *H. annuus* was also reduced with the increase in nickel treatment at 50, 100 and 150 ppm concentration. Nickel treatment at all concentration non-significantly affected the root / shoots fresh weight ratio for *H. annuus* seedlings. A significant effect on root / shoot dry weight ratio for *H. annuus* seedlings at 150 ppm concentration was observed. The biomass production of *H. annuus* seedlings was decreased with the increase in nickel treatment (Figure 4).

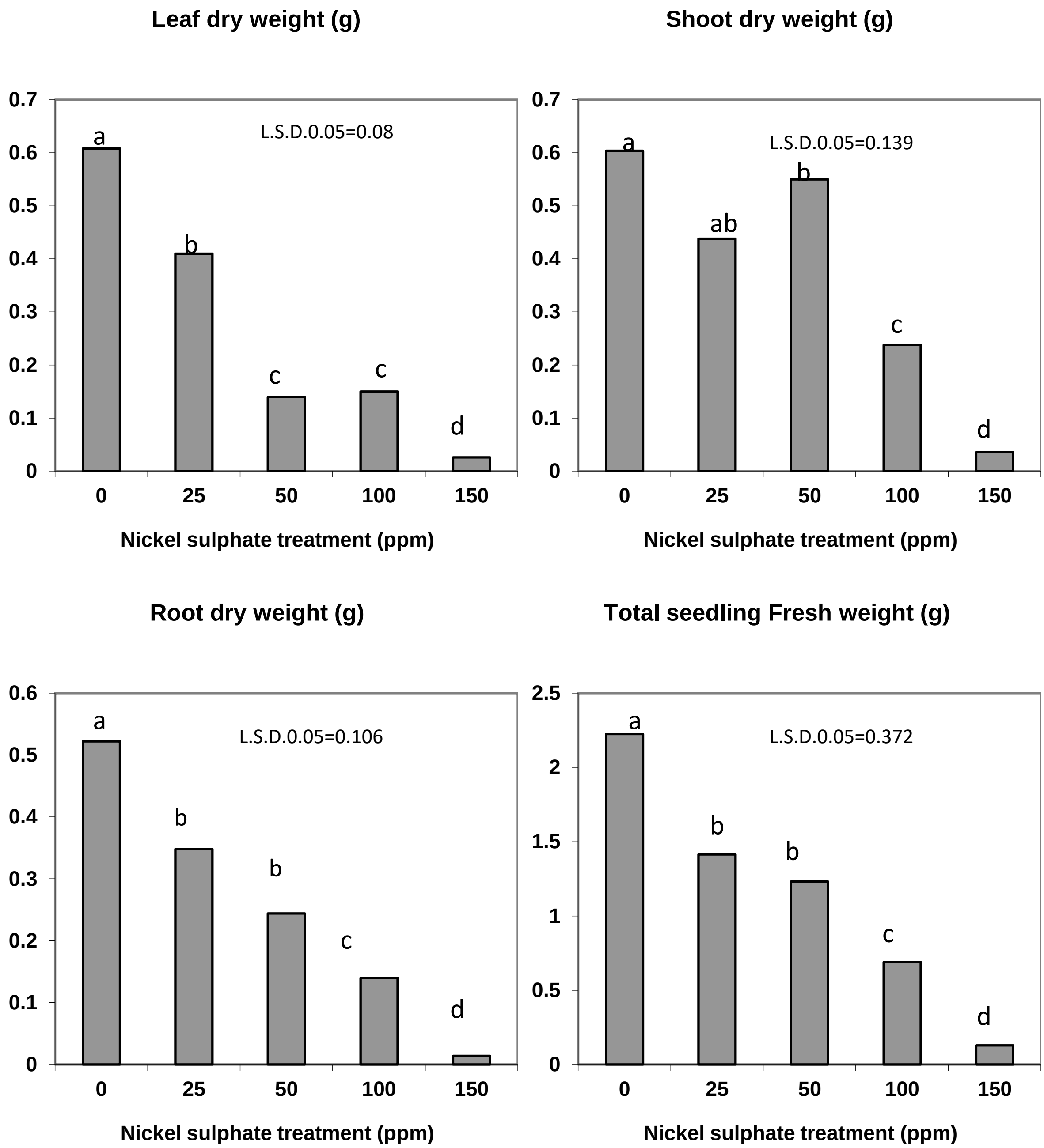

Figure 3 Effects of different concentration (0, 25, 50, 100, 150 ppm) of Nickel sulphate (Ni SO_4) on leaf, shoot, root dry weight and total seedling dry weight (g) of *Helianthus annuus*. Statistical significance determined by analysis of variance. Numbers followed by the same letters in the same bar not significantly different ($p<0.05$) according to Duncan's multiple range tests

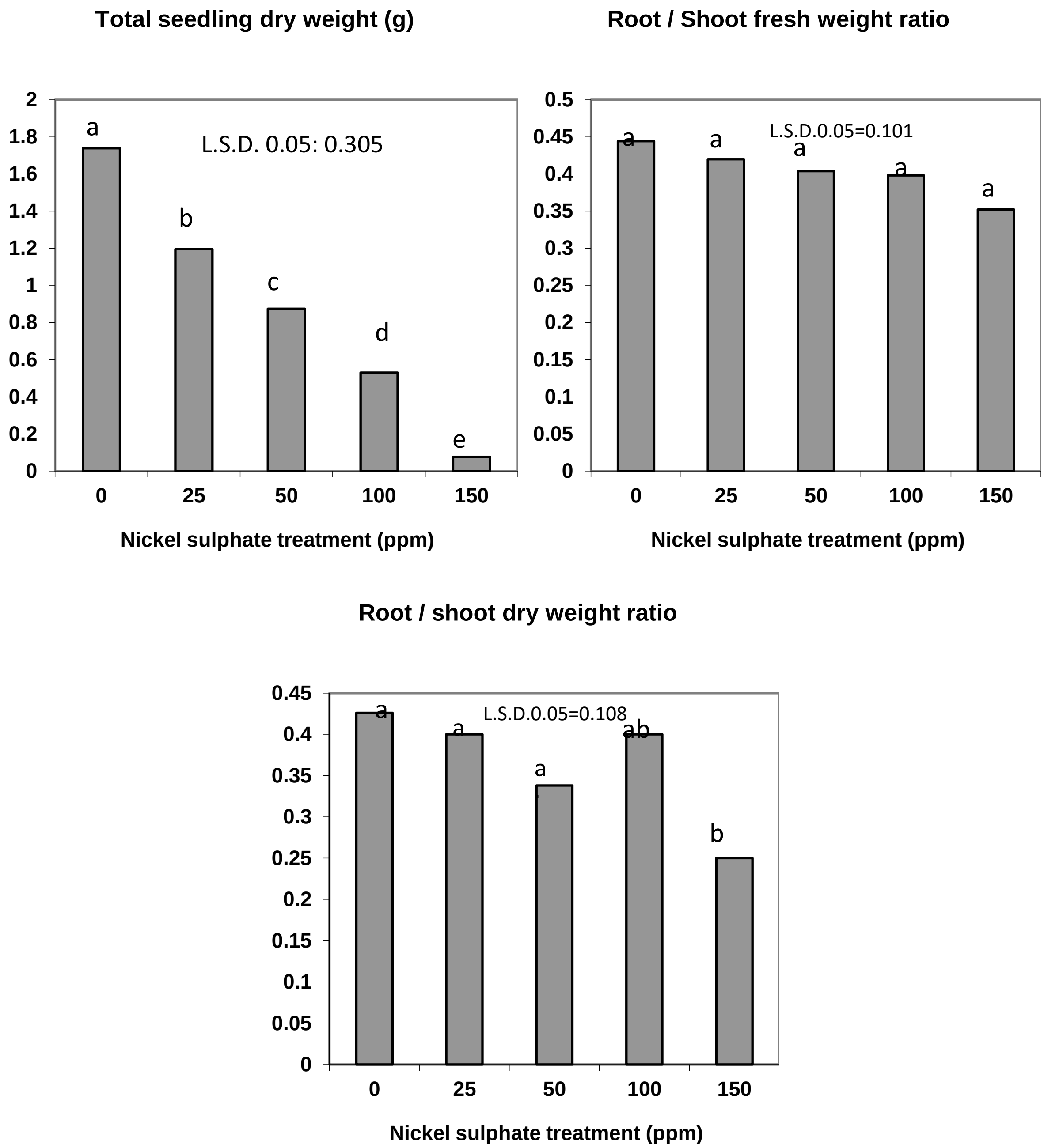

Figure 4 Effects of different concentration (0, 25, 50, 100, 150 ppm) of Nickel sulphate (Ni SO_4) on total seedling dry weight (g), root/shoot fresh weight ratio, root / shoot dry weight ratio of *Helianthus annuus.* Statistical significance determined by analysis of variance. Numbers followed by the same letters in the same bar not significantly different ($p<0.05$) according to Duncan's multiple range tests

Nickel treatment at 25 ppm showed significant ($p<0.05$) decrease in shoot and seedling length of *L. esculentum* (Figure 5). Seedling length, leaves numbers, leaf area and root / shoot growth performance of *L. esculentum* was further decreased with the nickel concentration treatment at 75 and 100 ppm. Nickel treatment at 25 ppm decreased shoot fresh weight of *L. esculentum* (Figure 6). Nickel treatment at 50 ppm significantly decrease leaves, root and total seedling fresh weight of *L. esculentum*. The effect of nickel treatment was found more in shoot than root growth for *L. esculentum* seedlings. The nickel treatment at 25 ppm significantly decreased root, shoot and leaves dry weight of *L. esculentum*.

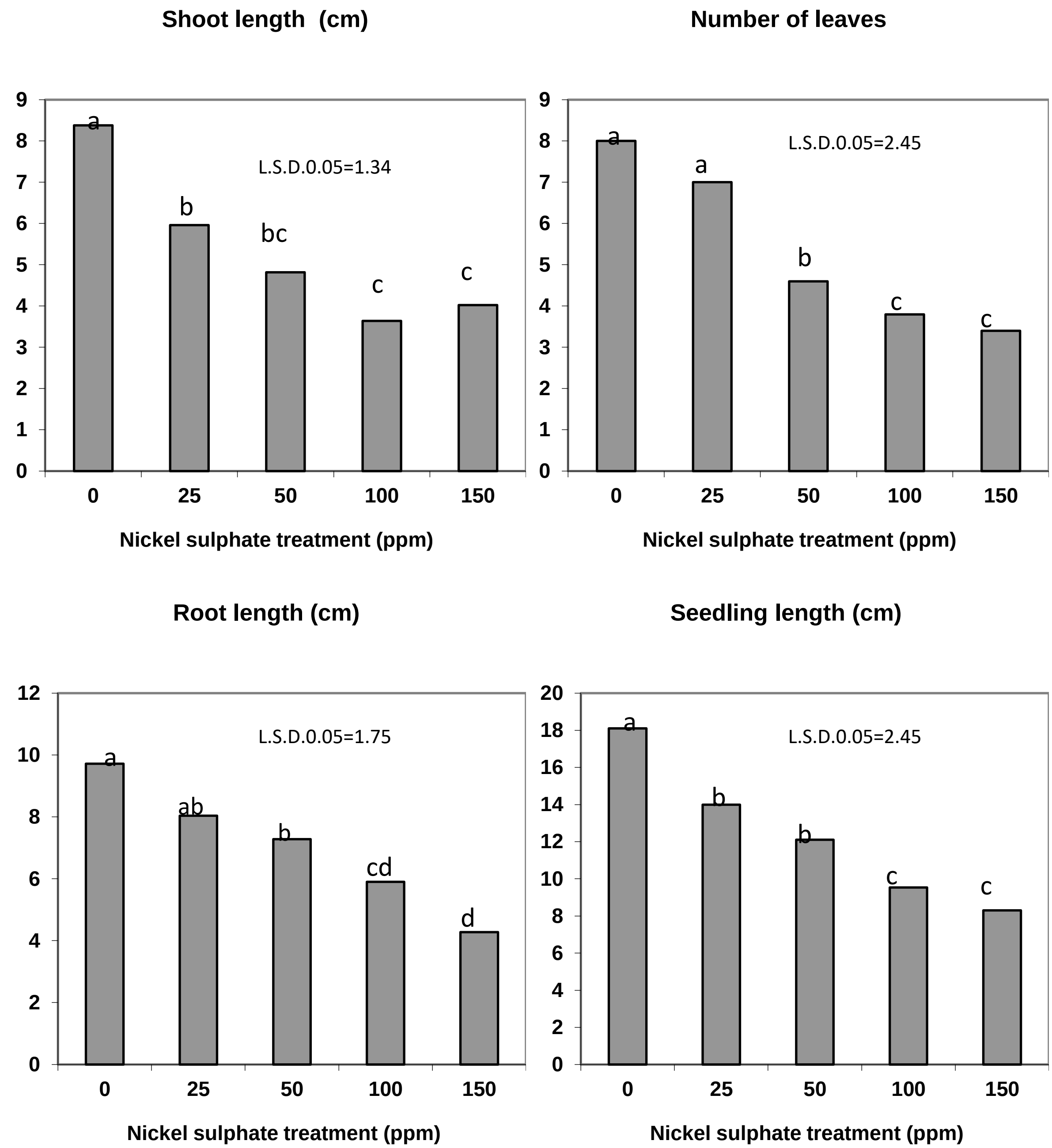

Figure 5 Effects of different concentration (0, 25, 50, 100, 150 ppm) of Nickel sulphate (Ni SO_4) on shoot, root, seedling length and number of leaves of *Lycopersicon esculentum.* Statistical significance determined by analysis of variance. Numbers followed by the same letters in the same bar not significantly different ($p<0.05$) according to Duncan's multiple range tests

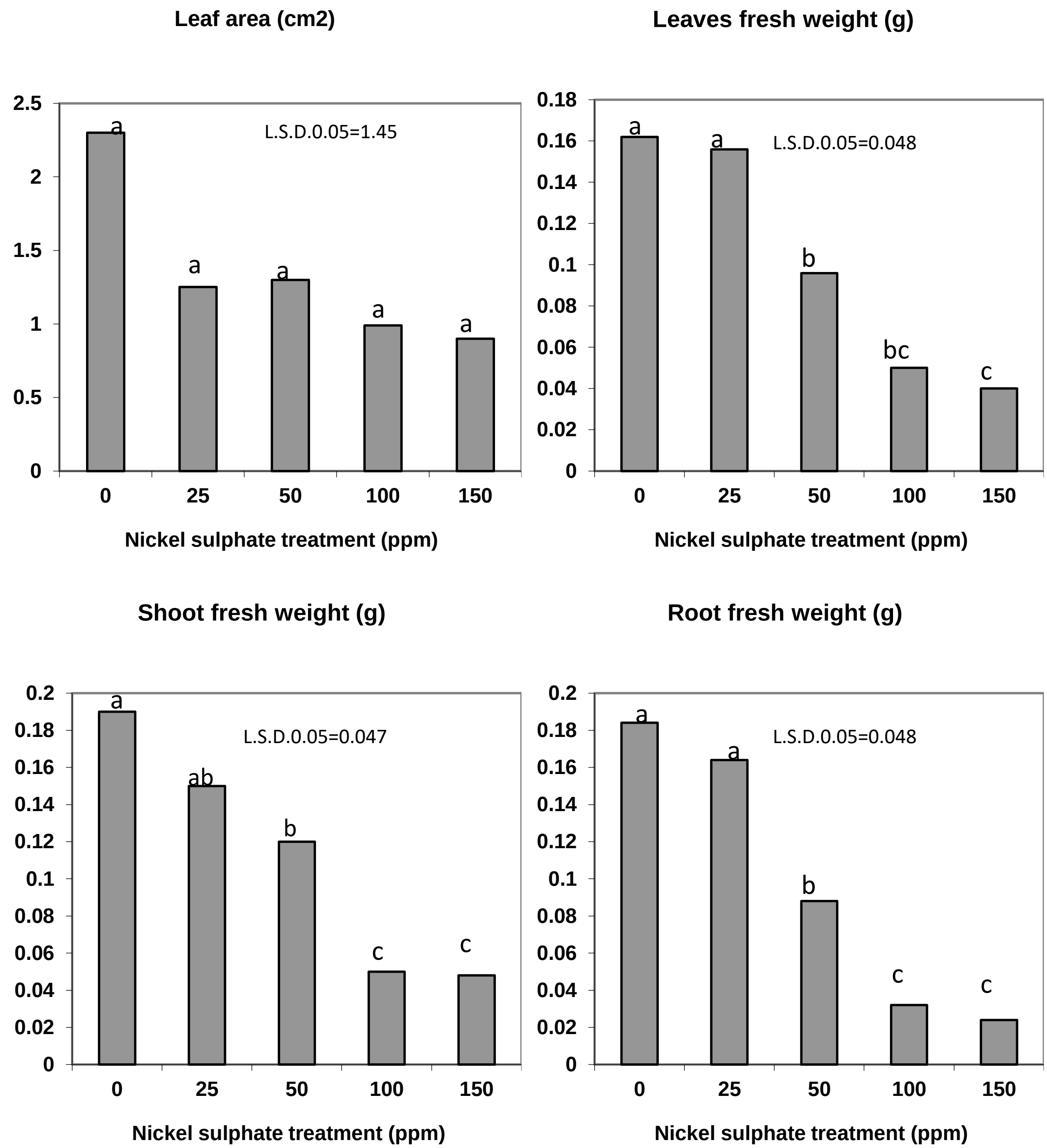

Figure 6 Effects of different concentration (0, 25, 50, 100, 150 ppm) of Nickel sulphate (Ni SO_4) on leaf area (cm^2), leaves, shoot and root fresh weight (g) of *Lycopersicon esculentum.* Statistical significance determined by analysis of variance. Numbers followed by the same letters in the same bar not significantly different ($p<0.05$) according to Duncan's multiple range tests.

Nickel treatment influenced on the seedling tolerance indices of *H. annuus* and *L. esculentum* as compared to control seedlings (Figure 8). The seedlings of *L. esculentum* and *H. annuus* showed lowest percentage of tolerance to nickel treatment at 150 ppm level. Lowest tolerance (44.03 %) to nickel treatment at 150 ppm in *L. esculentum* was observed. The seedling of *H. annuus* than *L. esculentum* was found good to nickel treatment.

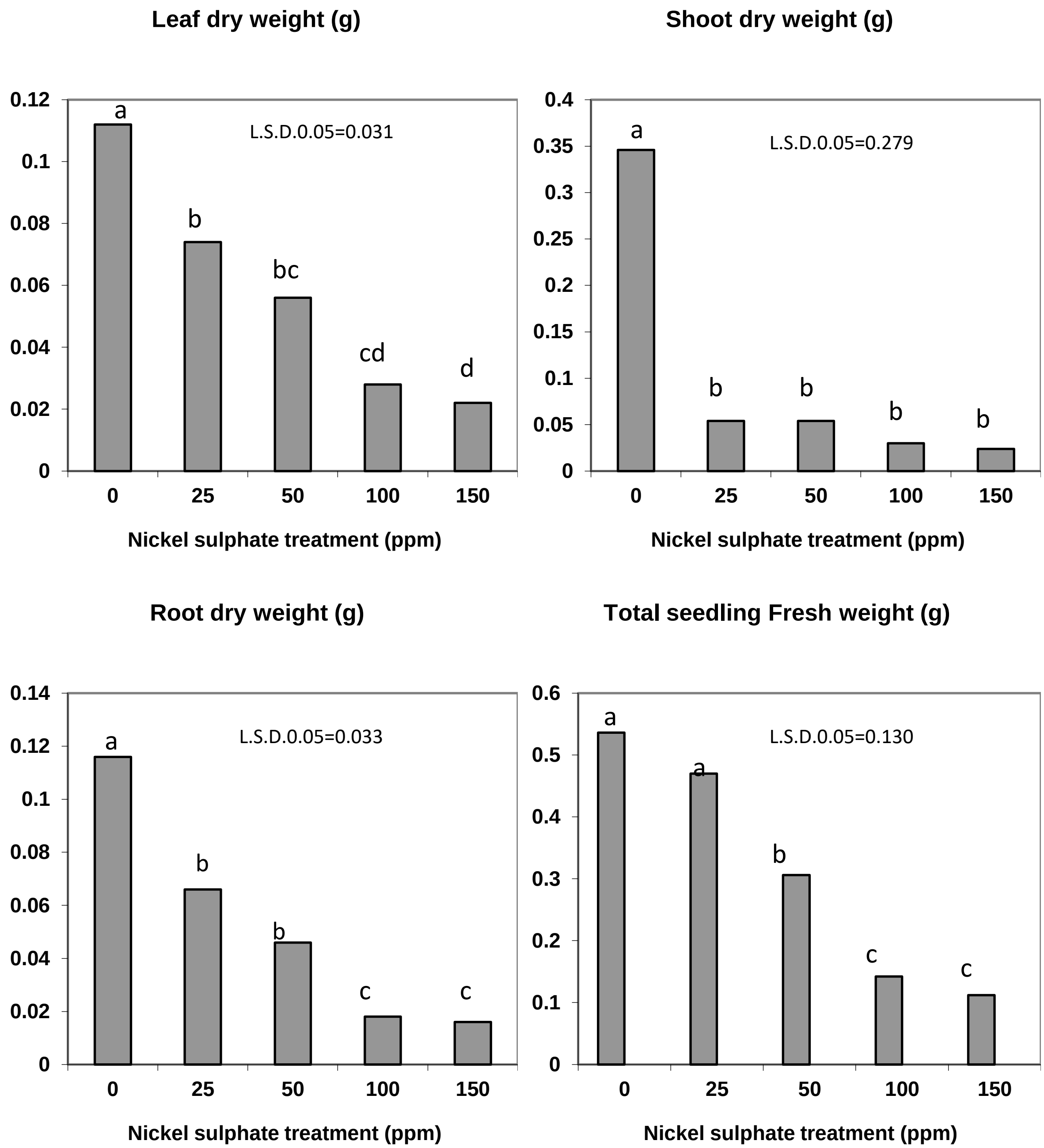

Figure 7 Effects of different concentration (0, 25, 50, 100, 150 ppm) of Nickel sulphate (Ni SO_4) on leaf, shoot, root dry weight and total seedling dry weight (g) of *Lycopersicon esculentum.* Statistical significance determined by analysis of variance. Numbers followed by the same letters in the same bar not significantly different ($p<0.05$) according to Duncan's multiple range tests

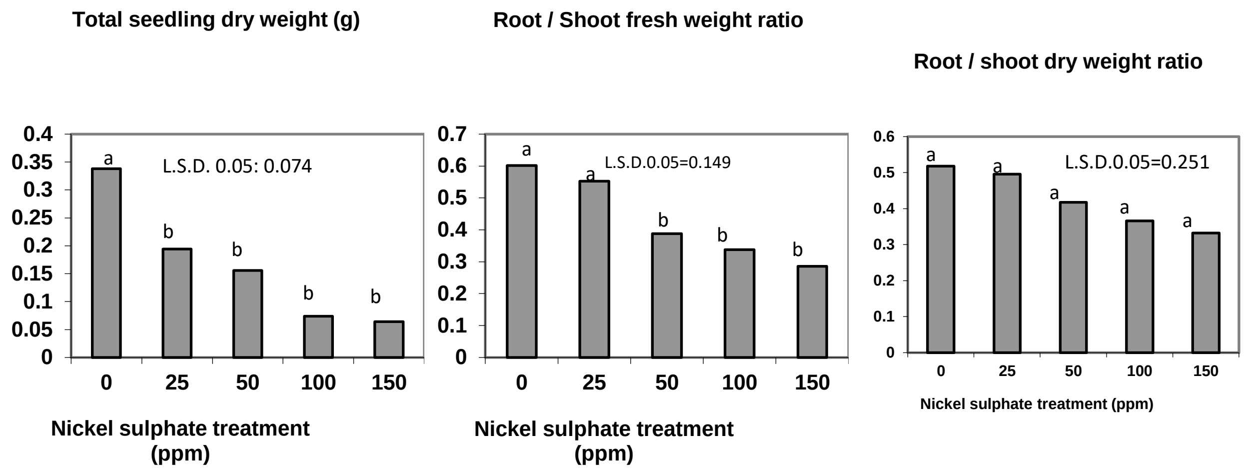

Figure 8 Effects of different concentration (0, 25, 50, 100, 150 ppm) of Nickel sulphate (Ni SO_4) on total seedling dry weight (g), root/shoot fresh weight ratio, root / shoot dry weight ratio of *Lycopersicon esculentum.* Statistical significance determined by analysis of variance. Numbers followed by the same letters in the same bar not significantly different ($p<0.05$) according to Duncan's multiple range tests

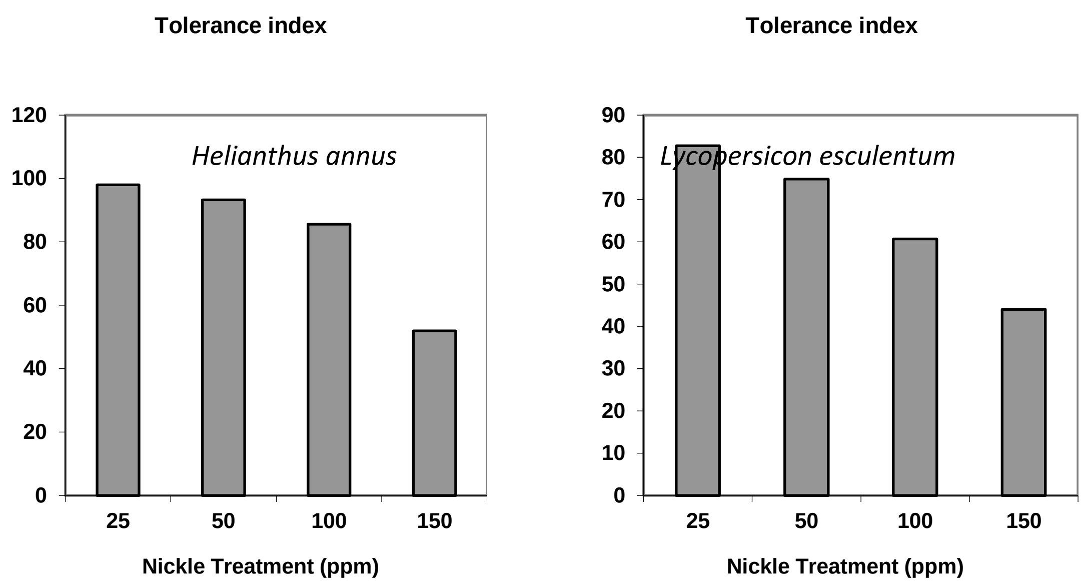

Figure 9 Tolerance index of *H. annuus* and *L. esculentum* seedling to different concentrations of Nickel as compared to control

4. DISCUSSION

The essential element cannot be substituted with any other nutrient or their absence cannot complete the life cycle of plant (Eskew et al., 1983; Andreeva et al., 2001). In this study, nickel stress affected the seedling growth and biomass of sunflower (*H. annuus*) and tomato (*L. esculentum*). The harmful effects of nickel on the metabolism of cabbage and inhibition of root branching in higher plants well documented (Pandey and Sharma, 2002; Seregin and Kozhevnikova, 2006). The pollutant stress changes the phenological behaviour and other growth characteristics on other plants. In an investigation the cellular redox imbalance produces oxidative stress in plants due to metal (Sharma and Dietz, 2009). Nickel treatment at 0, 25, 50, 100 and 150 ppm concentration responded differently to growth parameters. A slight decline in seedling height of sunflower and tomato at 25 ppm nickel treatment was observed.

The decreasing trend in seedling growth parameters was observed with the increase in metal concentration treatment. The similar types of nickel toxicity on the growth and structure of two Maize cultivars, *Zea mays* reported earlier by Huillier et al., (1996). The root development was found more sensitive to nickel than shoot of both crops. Nickel stress also had a variable effect on

biomass production of sunflower and tomato. Nickel also showed a decrease weight of root, shoot and leaves of both plant species. Nickel at concentrations above 50 ppm treatment decreased the seedling growth of sunflower and tomato. Similar sensitivity effects of Ni noticed in *Phaseolus vulgaris* and for other crop plants (Piccini and Malavolta, 1992; Krupa et al., 1993; Moya et al., 1993*)*. The more decline of root in both crops might be due to the high uptake of Ni from the medium and agreed with the work of Cataldo et al., (1978).

The reduction in seedling dry weight may be due to the disturbances in the photosynthesis activities and changes in chlorophyll and structure of chloroplast. The critical Ni concentration reduced the barley yield by 15% (Brown et al., 1987). The different responses to nickel toxicity may be result from the different metal bioaccumulation patterns in both plant species. Similarly, the growth inhibition in (*Coccinia indica* - Cucurbitaceae) and Mentha (*Mentha viridis* - Lamiaceae) due to nickel toxicity at 300 ppm was observed (Srinivas et al., 2013).

In visual observation the foliar injuries in leaves as chlorosis was also found in both plant species. The color of leaves o*f H. annuus* and *L. esculentum* become yellow at higher concentrations of Ni at 100 ppm. The visible symptom of nickel toxicity were initiated in younger leaved after 5 days treatment and the young expanding leaves exhibited interveinal chlorosis. Later on, shrinkage of apical margin of leaf lamina was observed in these leave. The region showed shrinkage and turned to brown in relative mature leaves. Later on, black dots were also developed in these lesions. The older leave developed necrosis from tip region and finally leaves turned brownish and were shed off. The observed visible effects like off-white lesions, chlorosis and necrosis on leaves due to excess nickel treatment in seedlings were in accordance with other workers.

The treatment of Ni in cabbage developed Ni toxicity symptoms after 15-17 days in the form of chlorosis (Pandey and Sharma, 2002). In present reports, the chlorosis were seen on the leaves when treated at higher concentrations of Nickel at 100 and 150 ppm and agreed with the findings of Dubey and Pandey concluded that chlorosis on the leaves of *Vigna mungo* appeared with 40-100 μM Nickel chlorides treatment. The toxicity and tolerance stress in plants has a great ecological concern. Excessive bioaccumulation of metals to different aerial parts through roots leading to disruption in physiological processes of plant resulting reduction in seedling growth of plants. Heavy metal treatment is responsible for inhibition of photosynthesis and chlorophyll formation (Kupper et al., 2002).

5. CONCLUSION

It was concluded that root growth was affected more than shoot growth in seedlings of both species (Figure 1, 2, 3, 4, 5, 6, 7, 8, 9). Similarly, high concentration of nickel at 150 ppm showed more harmful effects on tomato than sun flower seedlings. The seedlings of sunflower showed more tolerance to nickel than tomato. It is suggested that at present we should publish our findings in print media about the nature of toxicity of heavy metals likewise, nickel and its tolerance to other plant species. It is also recommended that we should not release metals into the environment freely as these are phytotoxic to living organisms. It can cause a variety of negative effects on seedling growth and physiological function such as changes in protein and nucleic acid synthesis. There is a need to build up phytotoxicity data base to determine toxic effect concentrations for the other plant species. The obtained information on the level of toxicity can be spread over through print media for researchers, nongovernmental organization and other civic agencies working for the betterment of the environment. The strict enforcement of environmental protection rules can save the immediate environment. This study also showed that vegetable crops have the ability and are suitable indicator of metal stress.

Informed consent

Not applicable.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Adriano DC. Trace Elements in Terrestrial Environments. Biogeochemistry, Bioavailability and Risks of Metals. Springer-Verlag, New York 2001.
2. Andreeva IV, Govorina VV, Vinogradova SB, Yagodin BA. Nickel in Plants. Agrokhimiya 2001; 3:82–94.
3. Brown PH, Welch RM, Cary EE. Nickel: A micronutrient essential for higher plants. Plant Physiol 1987; 85:801–803.
4. Cataldo DA, Garland TR, Wildung RE, Drucker H. Nickel in plants. II. Distribution and chemical form in soybean plants. Plant Physiol 1978; 62:566-570.
5. Cempel M, Nikel G. Nickel: A review of its sources and environmental toxicology. Pol J Environ Stud 2006; 15(3):375-382.
6. Chamon AS, Mondol MN, Faiz B, Rahman MH, Elahi SF. Speciation analysis of nickel in the soils of Tejgaon Industrial Area of Bangladesh. Bangladesh J Sci Ind Res 2009; 44(1):87-1 08.
7. Davis RD, Beckett PHT. Upper critical levels of toxic elements in plants. II. Critical levels of copper in young barley, wheat, rape, lettuce and ryegrass and of nickel and zinc in young barley and ryegrass. New Phytol 1978; 80:23-32.
8. Demirezen D, Aksoy A, Uruc K. Effect of population density on growth, biomass and nickel accumulation capacity of *Lemna gibba* (Lemnaceae). Chemosphere 2007; 66:553-557.
9. Eskew DL, Welch RM, Cary EE. Nickel: An essential micronutrient for legumes and possibly all higher plants. Science 1983; 222:621–623.
10. Fuentes I, Espadas-Gill F, Talavera-May C, Fuentes G, Santamaria JM. Capacity of the aquatic fern (*Salvinia minima* Baker) to accumulate high concentrations of nickel in its tissues and its effect on plant physiological processes. Aquat Toxicol 2014; 155:142-150.
11. Gerhardt KE, Huang X, Glick BR, Greenberg BM. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. Plant Sci 2009; 176:20–30.
12. Holmgren GGS, Meyer MW, Chaney RL, Daniels RB. Cadmium, lead, zinc, copper and nickel in agricultural soils of the United States of America. J Environ Qual 1993; 22:335-348.
13. Huillier LL, Auzac JD, Durand M, Michaud-Ferriere N. Nickel effects on two maize (*Zea mays*) cultivars: Growth, structure, Ni concentration and localization. Can J Bot 1996; 74:1547-155 4.
14. Khalid BY, Tinsley J. Some effects of nickel toxicity on rye grass. Plant Soil 1980; 55:139-144.
15. Khan Z, Fan X, Khan MN, Khan MA, Zhang K, Fu Y, Shen H. The toxicity of heavy metals and plant signaling facilitated by biochar application: Implications for stress mitigation and crop production. Chemosphere 2022; 308(Part 3):136466. doi: 10.1016/j.chemosphere.2022.136466
16. Krupa Z, Siedlecka A, Maksymiec W, Baszynski T. *In vivo* response of photosynthetic apparatus of *Phaseo*lus *vulgaris* L. to nickel toxicity. J Plant Physiol 1993; 142:664-21.
17. Kupper H, Setlik I, Spiller M, Kupper FC, Prasil O. Heavy metal induced inhibition of photosynthesis: Targets of in vivo heavy metal chlorophyll formation. J Phycol 2002; 38:429-441.
18. Liibben S, Sauerbeck D. The uptake and distribution of heavy metals by spring wheat. Water Air Soil Pollut 1991; 57-58:239-247.
19. Ma Y, Rajkumar M, Freitas H. Improvement of plant growth and nickel uptake by nickel resistant plant growth promoting bacteria. J Hazard Mater 2009; 166:1154–1161.
20. Moya JL, Ros R, Picazo I. Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. Photosynth Res 1993; 36:75-80.
21. Pandey N, Sharma CP. Effects of heavy metals Cu, Ni and Cd on growth and metabolism of cabbage. Plant Sci 2002; 163:753-758.
22. Pavlova D. Nickel effect on root cell division in *Plantago lanceolata* seedlings. Aust J Bot 2017; 65:446-452.
23. Pavlova1 D, Vila D, Vila K, Bani A, Xhaferri B. Effect of nickel on seed germination of *Alyssum* species with potential for phytomining in Albania. Fresenius Environ Bull 2018; 27(3):13 45-1352.
24. Piccini DF, Malavolta E. Effect of nickel on two common bean cultivars. J Plant Nutr 1992; 15:2343-2350.
25. Ruiz E, Rodríguez L, Alonso-Azcárate J. Effects of earthworms on metal uptake of heavy metals from polluted mine soils by different crop plants. Chemosphere 2009; 75:1035–1041.
26. Sen AK, Bhattacharyya M. Studies of uptake and toxic effects of Ni (II) on *Salvinia natans.* Water Air Soil Pollut 1994; 78:141–152.
27. Seregin IV, Kozhevnikova AD. Physiological role of nickel and its toxic effects on higher plants. Russ J Plant Physiol 2006; 53(2):257–277.
28. Sharma SS, Dietz K. The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 2009; 14(1):43-50.
29. Srinivas J, Purushotham AV, Murali KKVSG. The effects of heavy metals on seed germination and plant growth on *Coccinia, Mentha* and Trigonella plant seeds in Timmapuram, EG. District, Andhra Pradesh, India. Int Res J Environ Sci

2013; 2(6):20-24.
30. Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, Lie DV, Mench M. Phytoremediation of contaminated soils and groundwater: Lessons from the field. Environ Sci Pollut Res Int 2009; 16:765-794.
31. Zhang S, Shan XQ. Is the bioavailability index applicable for trace elements in different types of soil? Chem Speciation Bioavailability 2000; 12:117.
32. Zhu H, Jia Y, Cao H, Meng F, Liu X. Biochemical and histopathological effects of sub chronic oral exposure of rats to a mixture of five toxic elements. Food Chem Toxicol 2014; 71: 166-175.